Abstract

Proteins associated with metabolic pathways have received considerable attention in an effort to understand the molecular details of the complex reactions catalyzed *in vivo*. Enzymes belonging to these pathways have also been explored as potential targets for therapeutic intervention. The L-Lysine biosynthesis pathway in particular remains an attractive target for the design of new anti-microbial compounds. The rationale for this interest stems from the finding that the pathway for Lysine biosynthesis is only present in bacteria and is absent in humans. *m*-DAP/L-Lysine is an essential component of the bacterial cell wall. The L-Lysine biosynthesis pathway in bacteria is fairly diverse. Three major routes for the biosynthesis of *m*-DAP/L-Lysine are currently known. These are the succinylase, the acetylase and the dehydrogenase pathways. The results reported in this thesis are based on enzymes involved in the L-Lysine biosynthesis pathway of a nosocomial pathogen, *Staphylococcus aureus spp.* COL. The structural and biochemical characterization of three enzymes, Dihydrodipicolinate synthase (DapA), Dihydrodipicolinate reductase (DapB) and Succinyl diaminopimelate desuccinylase (DapE) provide a mechanistic rationale for the activity of these proteins. These studies reveal substantial differences in the regulatory mechanisms of these enzymes that could potentially be utilized for the design of inhibitors that specifically target this biosynthesis route.

This thesis is organized as follows:

Chapter 1 provides an introduction to the topic of this thesis. The first part of this chapter describes the characteristic features of *Staphylococcus aureus* and aspects relating to the identification, pathogenesis and genomic differences in *S. aureus* strains. The general features of the peptidoglycan layer of the bacterial cell wall including its structure and composition are also discussed. The second part of this chapter provides an overview of the Lysine biosynthesis pathway and details of the enzymes in this pathway.

Chapter 2 describes the structural and functional features of Dihydrodipicolinate synthase (DHDPS) also referred to as DapA. DHDPS catalyzes the first committed step of L-Lysine biosynthesis. Here we report the crystal structure of the native and

pyruvate complexes of *S. aureus* DHDPS. *S. aureus* DHDPS is a dimer, both in solution as well as in the crystal. The functional characterization of *S. aureus* DHDPS revealed that this enzyme is active as a dimer. This feature distinguishes the *S. aureus* enzyme from the *E. coli* homologue where a tetrameric quaternary arrangement is essential for the activity of this protein. A comparison between the native and pyruvate-bound structures also provides a structural basis for the ping-pong reaction mechanism of this enzyme whereby the catalytic triad is drawn closer to facilitate proton transfer upon pyruvate binding. It was also noted that unlike the *E. coli* homologue, *S. aureus* DHDPS is not feedback inhibited by lysine. The lack of feedback inhibition in *S. aureus* DHDPS could be attributed to a unique allosteric site. The different quaternary arrangement and a distinct allosteric pocket in this enzyme thus provide a structural template for the design of specific inhibitors for this enzyme.

Chapter 3 is based on preliminary studies of Dihydrodipicolinate reductase (DHDPR), encoded by the *dapB* gene. DHDPR catalyzes the second committed step in *m*-DAP/L-Lysine biosynthesis. The *dapB* gene encoding DHDPR was cloned and over-expressed in *E. coli*. Two variations of the recombinant protein were examined- one with a hexa-histidine tag at the C-terminus and the other without any tag. The recombinant DHDPR with the C-terminal hexa-histidine tag was purified by Ni-affinity chromatography and was subsequently crystallized. However, data sets collected on these crystals could not be examined further due to pronounced pseudo-translational symmetry and poor resolution. The recombinant DHDPR protein without an expression tag was purified by anion-exchange and size exclusion chromatography. Analytical gel filtration studies with recombinant DHDPR is consistent with a tetrameric quaternary arrangement of DHDPR subunits with a calculated molecular mass of 135 kDa. Diffraction data were collected to 3.3 Å resolution on crystals of apo DHDPR. These crystals belong to the C-centered monoclinic space group (C2) with unit cell parameters a = 63.17 Å, b = 78.91 Å, c = 128.38 Å and γ = 110.0°. Assuming two molecules in the asymmetric unit, the calculated Matthew's coefficient (V_m) was 2.32 $Å^3\ Da^{-1}$ and solvent content was 47.0 %. Molecular replacement (MR) trials with a model combining *E .coli* DHDPR structures (residues 1-106 of 1ARZ and 108-241 of 1DIH) as a search model resulted in a successful MR solution with two molecules in the asymmetric unit.

Chapter 4 is based on the structure and regulatory mechanism of DapE also referred to as Sapep. This enzyme belongs to the M20 family of proteases and is characterized by diverse substrate specificity and multiple functional roles. These include Succinyl diaminopimelate desuccinylase, a Mn^{2+}-dependent di-peptidase and a β-lactamase. The chemical reaction involved in all these functions is broadly similar and involves amide bond hydrolysis. In an effort to understand the structure and regulatory features of this enzyme, the structure of Sapep was determined both in a Mn^{2+}-bound form and in a metal-free (apo) form. A comparison between these structures revealed that large inter-domain movements potentially regulate the activity of this enzyme. These structures also revealed an additional regulatory mechanism wherein the inactive conformation is stabilized by a disulfide bond in the vicinity of the active site. Although these cysteines, Cys155 and Cys178 are not active site residues, the reduced form of this enzyme is substantially more active as a peptidase. The characterization of disulfide bond in the apo-form of the protein in solution by mass spectrometric studies and the requirement of a reducing agent for optimal catalytic activity of this protein suggests that the conformational features noted in crystal structures are also likely in solution. The structural and biochemical features of this enzyme thus provide a basis to rationalize the multiple functional roles of this protein with potential applications to MRSA-specific therapeutic strategies.

Chapter 5 provides a summary of the biochemical and structural data on the three enzymes of the L-Lysine biosynthesis pathway in *S. aureus spp.* COL. The emphasis of the discussion in this chapter is on features that are specific to these *S. aureus* enzymes. The latter part of this chapter is based on the scope of future studies in this area.

The appendix sections of this thesis are based on a project involving the catalytic domain of a receptor protein tyrosine phosphatase CRYP-2/cPTPRO.

Appendix-I includes the structure-function analysis of the catalytic domain of CRYP-2/cPTPRO. CRYP-2/cPTPRO is a receptor protein tyrosine phosphatase (PTP) that is selectively expressed in neurons and has been implicated in axon growth and guidance. The extracellular receptor domain of this protein has eight fibronectin

typeIII repeat regions while the intracellular region consists of a catalytic PTP domain. The crystal structure of CRYP-2 revealed two molecules of the catalytic domain in the asymmetric unit. The substantial buried surface area of this crystallographic oligomer suggested a homo-dimer of the catalytic domain. Solution studies however suggested that this protein is a monomer in solution based on the elution profile of CRYP-2 in a size exclusion chromatography experiment. The monomeric nature of CRYP-2 thus suggests that dimerization induced modulation of enzyme activity, such as that seen in RPTP-α where a helix-turn-helix segment of one monomer blocks the active site of the other, is not possible in the case of CRYP-2. Both monomers of CRYP-2 reveal a nitrate ion bound at the active site. An advantage provided by the crystallographic dimer of CRYP-2 was that it allowed us to visualize this protein with its active site lid (WPD-loop) in both the open and closed conformations. A structural comparison of CRYP-2 with other PTP's suggests that minor conformational rearrangements, as opposed to dimerization, could serve to regulate the activity of members of the type III family of RPTPs.

Appendix-II describes a project to examine the feasibility of utilizing mesoporous matrices of alumina and silica for the controlled inhibition of enzymatic activity. In these studies, we employed bare and functionalized mesoporous alumina and MCM48 silica to deliver *para*-nitrocatechol sulfate (*p*NCS), a potent competitive PTP inhibitor. *p*NCS was chosen as model inhibitor because of the ease of monitoring its release using UV-Visible absorption spectroscopy. CRYP-2 was used as model enzyme in this analysis. Inhibition of catalytic activity was examined using the sustained delivery of *para*-nitrocatechol sulfate (*p*NCS) from bare and amine functionalized MCM48 and Al_2O_3. Among the various mesoporous matrices employed, amine functionalized MCM48 exhibited the best controlled release of *p*NCS and inhibition of CRYP-2.

Appendix-3 incorporates additional methodologies and technical details that could not be included in the main body of the thesis.